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Decamethrin Metabolites from Oxidative, Hydrolytic, and Conjugative Reactions in Mice

Luis O. Ruzo,* Judith L. Engel, and John E. Casida

Mouse metabolism of orally administered decamethrin differs from previous findings with rats as follows: the feces contain less decamethrin and more monohydroxy and dihydroxyester metabolites; more acid moiety metabolites are hydroxymethyl derivatives and less alcohol moiety metabolites are phenolic compounds: metabolites in mouse but not rat excreta include 3-(2,2-dibromovinyl)-2-*trans*-hydroxymethyl-2-methylcyclopropanecarboxylic acid sulfate, 3-phenoxybenzaldehyde, 3-phenoxybenzyl alcohol and its glucuronide, glucuronides of 4′-hydroxy-3-phenoxybenzyl alcohol and 5-hydroxy-3-phenoxybenzoic acid, and 3-phenoxybenzoyltaurine. Intraperitoneal (ip) administration yields the same metabolites but in different ratios. Decamethrin is hydrolyzed in vitro by esterases in blood, brain, kidney, liver, and stomach preparations. Mice pretreated with piperonyl butoxide (PB) or *S,S,S*-tributyl phosphorotrithioate (DEF) metabolize decamethrin less readily than normal mice by oxidative or hydrolytic pathways, respectively. Equitoxic doses of decamethrin (twice the LD_{50}, 6–191 mg/kg) administered orally or ip with different vehicles or ip to PB- or DEF-treated animals yield similar levels (~0.5 ppm) of decamethrin in the brain. Severe poisoning symptoms result on introducing this level of decamethrin into the brain by direct injection.

Decamethrin [(S)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,-3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] (Figure 1), a highly potent synthetic pyrethroid insecticide (Elliott, 1977; Elliott et al., 1974), is metabolized by rats (Ruzo et al., 1978) and mouse liver microsomal enzymes (Shono and Casida, 1978; Shono et al., 1979) by pathways that include hydroxylation of the methyl group trans to the carboxyl group, hydroxylation at the 2′, 4′, and 5 positions of the alcohol moiety, and hydrolysis of the parent compound or its hydroxy derivatives. In rats, the phenolic metabolites are excreted as glucuronides and sulfates and the carboxylic acid metabolites as glucuronides and glycine conjugates (Ruzo et al., 1978). The cyano fragment is converted via HCN to SCN^-, which is temporarily localized in the stomach and skin prior to excretion, and small amounts of 2-iminothiazolidine-4-carboxylic acid which is excreted more rapidly (Ruzo et al., 1978). Analogous reactions (Casida et al., 1979) are established in rats for compounds with the unresolved alcohol moiety (i.e., α*RS*) esterified with α-(4-chlorophenyl)isovaleric acid (i.e., fenvalerate) (Ohkawa et al., 1979) or 2,2,3,3-tetramethylcyclopropanecarboxylic acid (Crawford and Hutson, 1977).

It is of interest to compare the metabolic fate of decamethrin in mice and rats since both species are important for acute and chronic toxicity studies and for safety evaluations. Mice are generally more susceptible than rats to pyrethroid poisoning (Miyamoto, 1976; Ruzo and Casida, 1977). Treatment of mice with the oxidase inhibitor piperonyl butoxide (PB) or the esterase inhibitor *S,S,S*-tributyl phosphorotrithioate (DEF) further increases their susceptibility to decamethrin (Soderlund and Casida, 1977).

This study compares decamethrin metabolism in mice with our earlier report (Ruzo et al., 1978) in rats. It also examines the significance of oxidative, hydrolytic, and conjugative pathways and the importance of brain decamethrin levels in the susceptibility of mice to decamethrin poisoning.

MATERIALS AND METHODS

Chromatography and Radiocarbon Analyses. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 20 × 20 cm chromatoplates with 0.25-mm layer thickness (EM Laboratories, Inc., Elmsford, NY) and the following solvent systems: BAW, 1-butanol–acetic acid–water (6:1:1); BE, benzene–ethyl acetate (6:1); BFE, benzene (saturated with formic acid)–ether (10:3), two developments; CE, carbon tetrachloride–ether (3:1); EFW, ethyl acetate–formic acid–water (35:2:2); EH, ether–hexane (1:1), three developments; EMW, ethyl acetate–methanol–water (2:1:1); HE, hexane–ether (4:1), three developments. R_f values for decamethrin derivatives are given in Table I. In referring to solvent systems for two-dimensional development, (BFE × CE) indicates development in the first direction with BFE and in the second direction with CE. Unlabeled standard compounds were detected first with UV light (254 nm) and then by spraying with either $PdCl_2$ (0.5% w/v in 12 N HCl) or phosphomolybdic acid (20% w/v in ethanol) and heating at 110 °C for up to 30 min. Procedures for radioautography, ^{14}C quantitation, and cochromatography of ^{14}C metabolites or their derivatives with unlabeled standards are given by Ueda et al. (1975).

Chemicals. Metabolite Designations and Standards. Metabolites are designated as shown in Figure 1, e.g., 4′-HO-dec and 4′-HO-PBacid are decamethrin and

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

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Table I. Thin-Layer Chromatographic Properties and Cochromatographic Identification of Decamethrin, Its Metabolites, and Their Derivatives

compd[a]	R_f values with indicated solvent systems[b]				
	Decamethrin and Ester Metabolites				
	BE	BFE	CE	EH	HE
decamethrin[c]	0.66	0.92	0.63	*0.89*	*0.59, 0.64*[d]
2'-HO-dec	*0.48, (0.57)*	*0.78 (0.93)*	0.41	*0.65, 0.68*[d]	0.18, 0.21
4'-HO-dec[c]	*0.41 (0.55)*	*0.72 (0.92)*	*0.33*	*0.51, 0.54*[d] (0.72, 0.74)[d]	0.11 (0.43, 0.47)[d]
5-HO-dec[e]	0.47	0.76	0.38	0.60	0.16
t-HO-dec	*0.39*	*0.61*	0.24	*0.36, 0.41*[d]	
4'-HO, *t*-HO-dec	*0.10*	*0.21*	0.07	*0.18*	
unk-ester 1		0.72	0.57		
unk-ester 2	0.34	0.51			
	Nonconjugated Metabolites from the Acid Moiety				
	BAW	BE	BFE	CE	EH
Br_2CA[c]	0.78	*0.27 (0.57)*	*0.73 (0.92)*	*0.42*	*0.71 (0.88)*
t-HO-Br_2CA	0.73	0.04 (0.17)	*0.29 (0.44)*	0.08	*0.16*
unk 1		0.24	0.45	0.19	0.39
	Nonconjugated Metabolites from the Alcohol Moiety				
	BAW	BE	BFE	CE	EH
PBalc		*0.28*	*0.56*	*0.25*	*0.50*
PBald		*0.61*	*0.85*	*0.57*	*0.75*
PBacid	0.76	*0.16 (0.58)*	*0.63 (0.86)*	*0.30 (0.59)*	*0.35*
4'-HO-PBalc	0.71	0.10	0.25	0.05	
5-HO-PBalc		0.10	0.22		
4'-HO-PBacid	0.71	*0.06 (0.26)*	*0.28 (0.49)*	*0.08*	
5-HO-PBacid			0.30	0.07	

Conjugated Metabolites and Their Derivatives					
	Acid Moiety			Alcohol Moiety[f]	
	BAW	EFW		BAW	EFW
Br_2CA-gluc[e]	0.47	0.26	PBalc-gluc[e]	0.40	0.25
Br_2CA-glycine	*0.62*	*0.40*	PBacid-gluc[e]	0.40	0.25
t-HO-Br_2CA-gluc[e]	0.41	0.12	PBacid-glycine	*0.58*	*0.35*
t-HO-Br_2CA-sulfate[e]	0.52	0.28	PBacid-taurine	*0.45*	*0.08*
unk-conj.	0.36	0.04	4'-HO-PBalc-gluc[e]	0.38	0.17
			2'-HO-PBacid-sulfate	*0.51*	*0.18*
			4'-HO-PBacid-gluc[e]	0.38	0.17
			4'-HO-PBacid-sulfate	*0.55*	*0.25*
			5-HO-PBacid-gluc[e]	0.38	0.17
			unk-conj.	0.29	0.03

[a] See Figure 1 and the Materials and Methods section for structures and abbreviations. [b] Italic values designate compounds identified by cochromatography with unlabeled standards or with analogous dichloro compounds (*t*-HO-dec, 4'-HO, *t*-HO-dec, and *t*-HO-Br_2CA) in the indicated solvent systems. Methylated derivatives are given in parentheses. Chromatographic positions of metabolites in the BAW and BFE solvent systems are illustrated in Figure 2. [c] Dichlorovinyl analogues give identical R_f values (BE, BFE, CE, and EH). [d] Two numbers refer to αS enantiomer followed by αR enantiomer of decamethrin and its hydroxy and methoxy derivatives. [e] Not available as standard from synthesis but tentatively identified as metabolite by criteria given in the Materials and Methods section. [f] SCN^- [R_f 0.52 (BAW), 0.29 (EMW)] was also detected as a metabolite and identified as its 4-nitrobenzyl thiocyanate derivative [R_f *0.37* (BE), *0.69* (BFE), *0.31* (EH)]. The cyano fragment also gives an unknown (unk 1) [R_f 0.43 (BAW), 0.08 (EFW)].

3-phenoxybenzoic acid, respectively, hydroxylated at the 4' position, Br_2CA is the acid moiety, *t*-HO refers to the trans position for a hydroxymethyl substituent relative to the carboxyl group, gluc is a glucuronide, conj is a conjugate, and unk is an unknown. Standard unlabeled compounds for tentative characterization of metabolites and their derivatives by cochromatography are described by Ruzo et al. (1977, 1978) and Unai and Casida (1977). 3-Phenoxybenzoyltaurine (PBacid-taurine) was kindly provided by D. H. Hutson (Shell Toxicology Laboratory, Sittingbourne, Kent, U.K.).

^{14}C Compounds. Roussel-Uclaf-Procida (Paris, France) provided decamethrin labeled in the dibromovinyl (^{14}Cv), benzylic ($^{14}C\alpha$), and cyano (^{14}CN) carbons (Figure 1) with specific activities of 5.0, 60.0, and 51.5 mCi/mmol, respectively. Each labeled preparation was checked by TLC (HE and EH) prior to each experiment and when necessary it was repurified (HE) to radiochemical purity >99%. The specific activity of [^{14}Cv]Br_2CA was 5.1 mCi/mmol.

Treatment of Mice, Collection and Analysis of Excreta, and Determination of Tissue Residues. Male albino mice (18–20 g; Simonsen Laboratories Inc., Gilroy, CA) were treated with [^{14}C]decamethrin or [^{14}C]Br_2CA dissolved in methoxytriglycol (MTG) or olive oil using the intraperitoneal (ip) or oral routes with 40- and 90-μL carrier vehicle, respectively. For oral administration, 100 μL of carrier solvent was used as a wash for the stomach tube. The doses of ^{14}C compounds are tabulated later with each individual study. In one series of experiments, mice were treated ip with PB at 150 mg/kg or DEF at 50 mg/kg and after 1 and 6 h, respectively, they were administered [$^{14}C\alpha$]decamethrin. Treated mice were held in all-glass metabolism cages for 3 or 8 days for collection of urine and feces during which time the $^{14}CO_2$ (if any) was collected for 2 days. The animals were sacrificed at 3 or 8 days after treatment and the residual ^{14}C in tissues was determined by combustion. These procedures are similar to those reported by Gaughan et al. (1977).

The reported data are average values for three mice at 3 days and two mice at 8 days after oral administration of the labeled compounds and for four mice at 3 days after ip administration of [$^{14}C\alpha$]decamethrin to each of normal

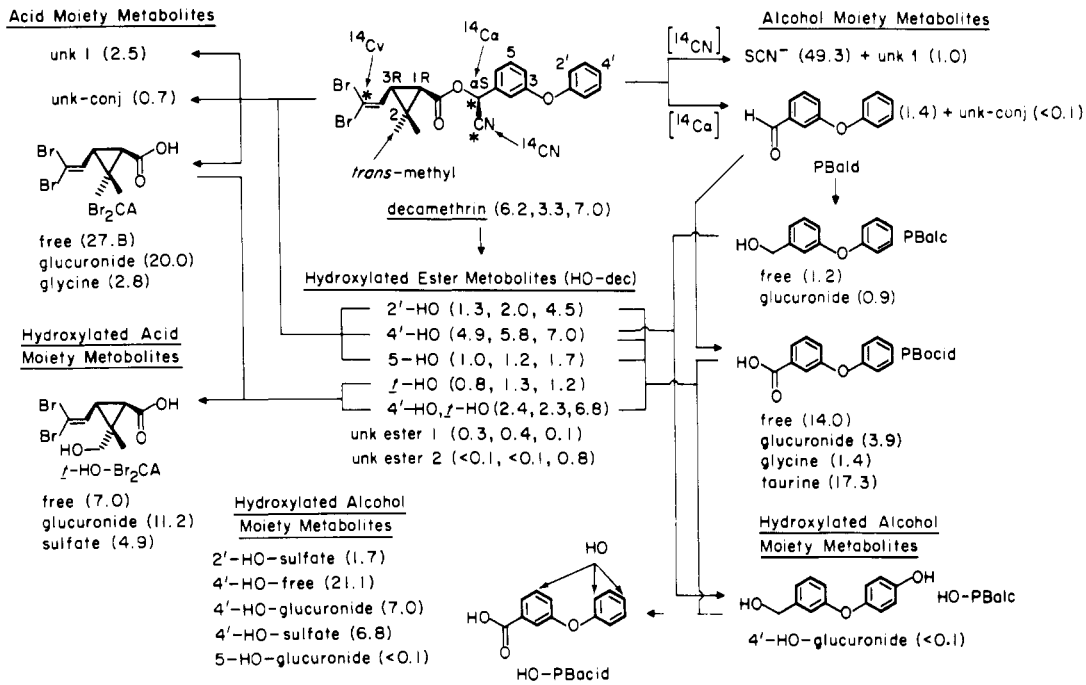

Figure 1. Metabolic pathways for decamethrin in mice. Metabolite yields are given in parentheses as percent of the orally administered dose in urine and the methanol extract of feces after 8 days. Yields for cleavage products are from experiments with the appropriate ^{14}C preparations and for esters are from the ^{14}Cv, $^{14}C\alpha$, and ^{14}CN preparations, respectively. See Table II for treatment conditions and ^{14}C balance.

mice and ones treated with PB or DEF. The total ^{14}C recovery values for excreta and tissues were 97 ± 3% (average and standard error for all analyses) of the administered dose. For convenience, they are normalized to 100%. Yields of fecal metabolites are corrected for cross-contamination (5–20%) from urinary metabolites. The correction factor was determined from the proportion of polar metabolites in feces that reproduced exactly the TLC metabolite pattern of the corresponding urine sample. Tissue residues are given as parts per billion (ppb) equivalents of administered ^{14}C compound based on fresh tissue weights.

For quantitation of individual metabolites, the urine was spotted directly for TLC and the feces was extracted with methanol and the methanol extract was analyzed using, in each case, the BAW or EFW solvent system in one-dimension or the BFE × CE and BFE × EH solvent systems in two dimensions. To minimize metabolite decomposition, appropriate unlabeled standards were added before concentration of the methanol extracts under N_2 (Shono et al., 1978).

Decamethrin and Metabolites in Tissues and in Vitro Metabolism. [$^{14}C\alpha$]- and [^{14}Cv]decamethrin were orally administered to two mice each at 3.1 and 3.6 mg/kg, respectively, and 6 h later the animals were sacrificed. The blood, kidney, liver, and stomach were individually homogenized in methanol. The methanol-soluble fraction following centrifugation was analyzed by TLC (BE, BFE). Methanol extracts of stomach were also analyzed 3 and 8 days after oral treatment with [^{14}CN]decamethrin at 1.1 and 2.2 mg/kg, respectively.

Brain levels of decamethrin were determined following [$^{14}C\alpha$]decamethrin administration involving various routes and carrier solvents. The doses administered were ca. two times the 24-h LD_{50} values. Brains removed from mice at the time of death (1.5–5 h) or on sacrifice at early but definite symptoms of pyrethroid poisoning (0.3–1.5 h) were extracted with methanol and analyzed as above.

For in vitro metabolism studies, the tissues were homogenized at 20% (w/v) in 0.8% NaCl solution and the homogenate was incubated with 3 μg of [$^{14}C\alpha$]decamethrin at 37 °C for 2 h. These incubations were made in the presence and absence of 2.5×10^{-3} M tetraethyl pyrophosphate (TEPP), an esterase inhibitor. Centrifugation of the homogenate followed by rehomogenization of the particulate fraction in methanol yielded an aqueous fraction and methanol extract with >80% ^{14}C recovery for individual TLC analysis (BE, BFE).

Tentative Characterization of Metabolites. Individual metabolites, separated as above and detected by radioautography, were recovered from the appropriate gel regions by extraction with methanol. Esters were recognized by their identical chromatographic properties with the three labeled preparations. Compounds directly cochromatographed with authentic standards are shown in Table I. Three decamethrin metabolites (*t*-HO-dec, 4'-HO, *t*-HO-dec, and *t*-HO-Br_2CA) are tentatively identified by cochromatography with analogous dichlorovinyl esters or dichlorovinyl acids [for justification, see Ruzo et al. (1978) and Table I].

Ester metabolites indicated as 4'-HO- and 5-HO-dec were cleaved with $NaBH_4$ (Ruzo et al., 1978) and the resulting alcohols cochromatographed with authentic 4'-HO- and 5-HO-PBalc, respectively. *t*-HO-Dec was also treated with $NaBH_4$ and a cleaved product was oxidized with $KMnO_4$ (Ruzo et al., 1978) and cochromatographed with PBacid.

Compounds designated as glucuronides and sulfates were subjected to enzymatic or HCl cleavage as previously reported (Ruzo et al., 1978) and cochromatography of the cleavage products as specified in Table I. Glucuronidase cleavage products of 4'-HO-PBalc-glu and 5-HO-PB-acid-gluc were cochromatographed with the appropriate aglucons in solvent systems BE, BFE, and CE, and BFE and CE, respectively. *t*-OH-Br_2CA-sulfate cochromatographs after sulfatase cleavage with *t*-HO-Cl_2CA before methylation (BFE × EH) and with its methyl ester after treatment with diazomethane (BFE × BE). Thiocyanate was identified, as before (Ruzo et al., 1978), by conversion to *p*-nitrobenzyl thiocyanate.

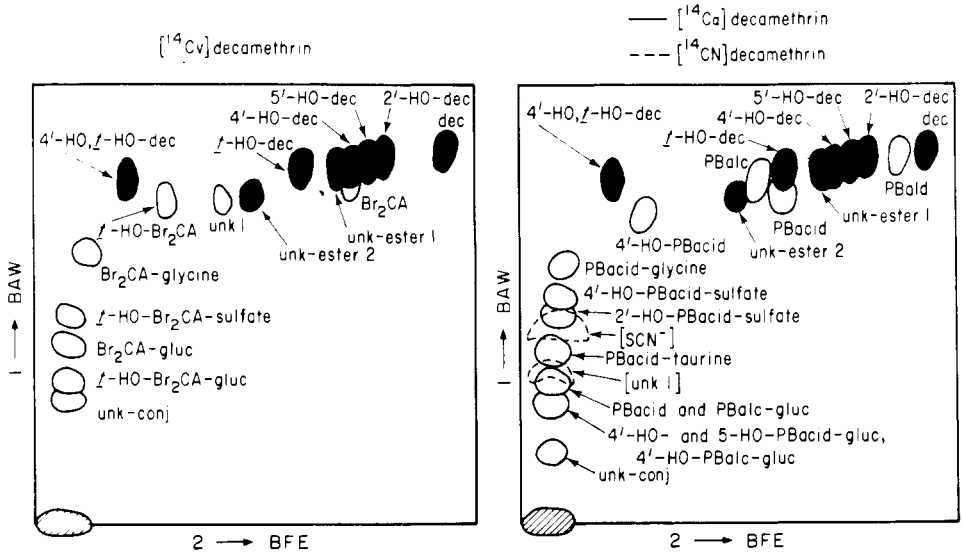

Figure 2. ^{14}C-labeled compounds in the urine and the methanol extract of feces of mice 8 days after oral administration of three labeled preparations of decamethrin as resolved by two-dimensional TLC. The solvent fronts are the appropriate outlines of the figures. The origin, which contains no ^{14}C compounds, is indicated at the lower left of each figure. Decamethrin and ester metabolites detected with all labeled preparations are shown by solid circles. Open circles indicate metabolites detected with only one of the ^{14}C preparations. Metabolites designated as "unk" are unidentified. Figure 1 indicates the structures of the compounds and the quantitative data on excreted products.

Toxicity Determinations. Approximate LD_{50} values for decamethrin were determined following oral and ip administration in MTG and olive oil, sometimes using animals pretreated with PB or DEF as above. Intracerebral toxicity was evaluated by injecting decamethrin dissolved in ethylene glycol (1–3 μL) according to Rainsford (1978).

RESULTS

Distribution, Metabolism and Excretion of Orally Administered [^{14}Cv]-, [^{14}Cα]- and [^{14}CN]Decamethrin. On oral administration of [^{14}Cv]- or [^{14}Cα]decamethrin, the ^{14}C is rapidly and almost completely excreted with little tissue retention after 8 days (Table II). The highest decamethrin equivalents from these labeled preparations appear in fat. These findings contrast to those with [^{14}CN]decamethrin where the ^{14}C is excreted much slower and the highest residues appear in skin and stomach.

Chromatographic patterns of the excreted decamethrin metabolites 8 days following oral administration are shown in Figure 2 and their yields are given in Figure 1. Results of the ^{14}C balance study and the amounts of metabolites in the excreta 3 days after oral administration of [^{14}Cα]decamethrin are given in Table III.

The feces but not the urine contain unmetabolized decamethrin, four monohydroxy ester metabolites (2′-HO-, 4′-HO-, 5-HO-, and *t*-HO-dec) and one dihydroxy ester metabolite (4′-HO,*t*-HO-dec) (Figure 1). Decamethrin and 4′-HO-dec are detected not only as the administered αS epimer but also in part as the αR epimer (Table I, footnote *d*). Two unknown ester metabolites (unk esters 1 and 2) appear in low yields with all ^{14}C preparations. The feces also contain appreciable amounts of ester cleavage products which are not conjugated.

Decamethrin metabolites from the acid moiety are mostly Br_2CA, *t*-HO-Br_2CA, and their glucuronide conjugates. There are also smaller amounts of Br_2CA-glycine and *t*-HO-Br_2CA-sulfate. *t*-HO-Br_2CA, both free and liberated on sulfatase cleavage of its conjugate, is tentatively identified by its chromatographic properties compared to the *trans*- and *cis*-hydroxymethyl derivatives of the dichlorovinyl acid (Table I, footnote *b*); this isomer assignment is further supported by its failure to lactonize upon acid treatment and by analogy with the metabolism of (1*R*)-*cis*-permethrin (Gaughan et al., 1977). An unidentified metabolite ([^{14}Cv]unk 1), detected after 8 but not 3 days, cochromatographs with the dibromovinyldimethylcyclopropylmethyl alcohol derivative of Br_2CA and is oxidized by $KMnO_4$ to a product cochromatographing with Br_2CA; however, the metabolite derivative formed on reaction with phenyl isocyanate does not cochromatograph with the anticipated *N*-phenylcarbamate. A polar un-

Table II. Radiocarbon in the Urine, Feces, and Tissues of Mice up to 8 Days after Oral Administration of [^{14}Cv]-, [^{14}Cα]-, and [^{14}CN]Decamethrin[a]

sample analyzed	labeling position ^{14}Cv	^{14}Cα	^{14}CN
Administered Dose, mg/kg	4.4	1.7	2.2
% of Administered Dose[b]			
urine			
0–1 day	28.9	40.5	13.1
1–2 days	13.8	5.0	8.5
2–5 days	12.0	15.7	10.3
5–8 days	2.6	3.9	3.6
feces			
methanol extract			
0–1 day	19.0	18.6	20.8
1–2 days	9.5	4.0	16.4
2–5 days	7.2	5.1	4.5
5–8 days	0.9	0.4	2.2
unextractable, 0–8 days	5.1	5.8	13.8
carcass and tissues, 8 days	1.0	1.0	6.8
Tissue Residue at 8 Days, ppb of Decamethrin Equiv[c]			
blood	4	5	54
brain	17	0	4
fat	273	115	79
kidney	39	27	20
liver	47	20	19
skin	77	3	778
stomach	28	9	175

[a] Values at 3 days are given in Table I of the microfiche supplement to this report. [b] $^{14}CO_2$ values are <0.1% for 0-2-day samples with each labeled preparation. [c] Comparable values for bone, heart, intestine, lung, muscle, spleen, and testes are <60 ppb.

Table III. Distribution, Metabolism, and Excretion of [^{14}Cα]Decamethrin 3 Days after Oral or Intraperitoneal Administration to Normal Mice or Mice Pretreated with Piperonyl Butoxide (PB) or Tributyl Phosphorotrithioate (DEF)

compound or sample[a]	treatment schedule[b] oral[c]	ip	ip (PB)	ip (DEF)
	% of Administered ^{14}C in Excreta at 0–3 Days[d]			
decamethrin	9.2	1.6	0.3	1.3
nonhydroxylated metabolites				
PBalc	0.1	3.0	4.2	0.8
PBald	1.2	1.3	0.4	0.6
PBacid	0.8 (2.5)	6.4 (26.4)	7.2 (29.4)	3.8 (14.1)
PBalc- + PBacid-conj.	(31.3)	(13.2)	(20.6)	(16.6)
total	35.9	50.3	61.8	35.9
hydroxylated metabolites				
esters	14.2	14.3	10.0	16.6
4′-HO-PBacid	2.7 (8.9)	5.9 (9.1)	0.7 (0.6)	5.3 (0.3)
5-HO-PBacid	0.0	0.7	0.2	1.1
HO-PBalc- + HO-PBacid-conj.	(12.7)	(4.3)	(5.3)	(11.1)
total	38.5	34.3	16.8	34.4
others				
unextractable, feces	9.2	10.8	13.4	18.6
carcass and tissues	6.1	3.0	7.7	9.8
	Tissue Residue at 3 Days, ppb Decamethrin Equiv			
fat	314	817	1686	3976
liver	33	56	56	99
brain	4	8	15	20

[a] The identity and yield of each metabolite and the overall excretion rate are given in Tables I and II of the microfiche supplement to this report. [b] Decamethrin was administered orally or ip at 1.2 or 1.1 mg/kg, respectively. [c] Two additional unidentified metabolites total 1.1% of the administered ^{14}C. [d] Metabolites in urine are indicated in parentheses. ^{14}C excretion percentages in feces at 24 and 48 h, respectively, are as follows: 18.0, 7.7 (oral); 19.8, 8.3 (ip); 14.2, 5.2 (ip, PB); 16.1, 7.6 (ip, DEF). ^{14}C excretion percentages in urine at 24 and 48 h, respectively, are as follows: 34.1, 17.6 (oral); 40.2, 6.8 (ip); 27.4, 12.6 (ip, PB); 21.5, 5.1 (ip, DEF).

known ([^{14}Cv]unk-conj), detected at low levels in urine, chromatographs (BAW) at lower R_f than the other metabolites and is not cleaved by acid (6 N HCl, 37 °C, 24 h), glucuronidase or sulfatase. Administration of Br_2CA to mice results in formation of the same products as [^{14}Cv]decamethrin except that unk 1 is not detected.

A major alcohol moiety metabolite is the taurine conjugate of PBacid which appears only in the urine. Other major metabolites are PBacid and 4′-HO-PBacid and its glucuronide and sulfate. Lower yields are obtained for PBald, PBalc and its glucuronide, PBacid-gluc, PBacid-glycine, 4′-HO-PBalc-gluc, 5-HO-PBacid-gluc, and probably 2′-HO-PBacid-sulfate. The finding of 2′-HO-dec in feces supports the suggestion that this sulfate is that of 2′-HO-PBacid. There is one minor unknown ([^{14}Cα]-unk-conj) chromatographing (BAW) below 4′-HO-PBacid-gluc. Compounds not present in the excreta either free or conjugated are other HO-PBacid and HO-PBalc derivatives reported by Unai and Casida (1977) or Ruzo et al. (1978).

The urine from [^{14}CN]decamethrin administration contains only two products, S^{14}CN$^-$ and a minor unknown of greater polarity which does not cochromatograph with 2-iminothiazolidine-4-carboxylic acid.

Effects of Piperonyl Butoxide (PB) and Tributyl Phosphorotrithioate (DEF) on Distribution, Metabolism, and Excretion of ip-Administered [^{14}Cα]-Decamethrin. PB and DEF reduce the rate of urinary ^{14}C excretion without altering the rate of fecal excretion of ^{14}C products derived from [^{14}Cα]decamethrin (Table III). The unextractable residue in feces and ^{14}C retention in the carcass and tissues after 3 days are greatest for DEF-treated mice, followed by those treated with PB. Decamethrin equivalents in fat are increased by two- and fivefold on pretreating the mice with PB and DEF, respectively.

The same [^{14}Cα]decamethrin metabolites are detected after ip (Table III) or oral (Table III and Figure 1) administration, but there is less excretion of unmetabolized decamethrin and PBacid conjugates (mostly the taurine derivative) after ip treatment. DEF decreases the hydrolysis products (nonhydroxylated metabolites) relative to controls while PB decreases the oxidation products (hydroxylated metabolites) (Table III). For example, PBacid is recovered in 32.8% yield from the controls and only 17.9% with DEF-treated mice, whereas 4′-HO-PBacid is in 15.0% yield with controls and only 1.3% with PB-treated mice.

Brain Levels of [^{14}Cα]Decamethrin and Its Metabolites. [^{14}Cα]Decamethrin administered orally or ip in different carriers and with PB or DEF or no pretreatment, so as to give a wide range of equitoxic doses (6–191 mg/kg), results in comparable levels (~0.5 ppm) of the pyrethroid in the brain, both at the times of poisoning symptoms and of death (Table IV). Intracerebral injection of decamethrin gives severe poisoning symptoms at 0.1–0.3 ppm relative to total brain weight and an LD_{50} value of 0.3–1 ppm on the same basis.

Analyses (TLC, BE, BFE) of the brain methanol extracts revealed PBald, PBalc, and PBacid in addition to decamethrin itself. PB and DEF increase the percentage of the ^{14}C in the brain due to decamethrin.

Metabolites in Other Tissues And in Vitro Metabolism. ^{14}C-Labeled compounds detected in blood, kidney, liver and stomach 6 h after oral administration of [^{14}Cα]- and [^{14}Cv]decamethrin are given in Table V. Decamethrin, Br_2CA and PBacid are detected in all cases. PBald appears in blood and kidney, PBalc appears in blood, and there is a trace amount of [^{14}Cv]unk-1 in the stomach. Other metabolites in liver and kidney are those also found in the urine.

Essentially all of the ^{14}C in the stomach at 3 or 8 days after oral administration of [^{14}CN]decamethrin is S^{14}CN$^-$ analyzed as *p*-nitrobenzyl thiocyanate.

[^{14}Cα]Decamethrin incubated with tissue homogenates yields PBald (for blood, brain, and kidney) and PBacid

Table IV. Brain Levels of [^{14}Cα]Decamethrin and Its Metabolites at Times of Poisoning Symptoms and Death after Oral or Intraperitoneal Administration of Equitoxic Decamethrin Doses in Two Carrier Vehicles to Normal Mice or Mice Pretreated with Piperonyl Butoxide (PB) or Tributyl Phosphorotrithioate (DEF)

treatment			brain decamethrin equiv, ppm[b]	
dose, mg/kg[a]	pretr. and carrier	route	poisoning symptoms	death
6	PB, MTG	Ip	0.58 (0.65 ± 0.18)	0.56 (0.75 ± 0.16)
15	DEF, MTG	Ip	0.43 (0.55 ± 0.12)	0.51 (0.68 ± 0.09)
30	MTG	Ip	0.30 (0.95 ± 0.25)	0.50 (1.20 ± 0.34)
54	olive oil	oral	0.26 (0.72 ± 0.16)	0.53 (1.21 ± 0.15)
61	MTG	oral	0.54 (1.04 ± 0.19)	0.74 (1.40 ± 0.15)
191	olive oil	Ip	0.17 (0.55 ± 0.09)	0.28 (1.08 ± 0.38)

[a] Doses are approximately twice the LD_{50} values under the indicated treatment conditions. [b] Results are average values for six mice in each of two separate experiments. The first values are for decamethrin per se while those in parentheses are for decamethrin plus metabolites which include PBald, PBalc, and PBacid.

Table V. ^{14}C-Labeled Compounds in Blood, Kidney, Liver, and Stomach of Mice 6 h after Oral Administration of [^{14}Cα]- and [^{14}Cv]Decamethrin at 3.1 and 3.6 mg/kg, Respectively

	metabolites detected with indicated labeling position	
tissue[a]	^{14}Cα	^{14}Cv
blood	PBald, PBalc, PBacid	Br_2CA
kidney	PBald, PBacid and its taurine conjugate, 4′-HO-PBacid-sulfate	Br_2CA, Br_2CA-gluc
liver	PBacid and its taurine, glycine, and glucuronide conjugates; 4′-HO-PBacid and its glucuronide and sulfate conjugates	Br_2CA, Br_2CA-gluc
stomach	PBacid	Br_2CA, unk 1

[a] Decamethrin is detected in all four tissues.

(for kidney, liver, and stomach). TEPP added to these homogenates drastically decreases the extent of metabolism in all tissues except stomach.

DISCUSSION

Decamethrin metabolism in mice involves four sites of oxidative attack (trans methyl group of the acid moiety and 2′, 4′, and 5 positions of the alcohol moiety), hydrolysis, and a variety of conjugation processes (Figure 1). Mice excrete less unmetabolized decamethrin than rats (Ruzo et al., 1978), suggesting a more efficient absorption and/or metabolism. Whereas rats hydroxylate decamethrin predominantly at the 4′ position, mice produce considerable amounts of the *t*-, 2′- and 5-hydroxy derivatives. Portions of the decamethrin and 4′-HO-dec are detected as the α*R* epimers probably due to an artifact resulting from racemization by exchange of the α proton with methanol (Ruzo et al., 1977, 1978) during sample handling.

The acid moiety is rapidly excreted as the glucuronide with smaller amounts free and as the glycine conjugate. The trans hydroxymethyl derivative is also excreted free, as the glucuronide and as the sulfate conjugate, the latter not detected in rats. Methyl hydroxylation is much more extensive following decamethrin than Br_2CA administration, indicating that it takes place in the most part before ester cleavage.

Decamethrin hydrolysis yields phenoxybenzylcyanohydrin (Shono et al., 1979) which readily degrades to PBald and HCN (Ruzo et al., 1977). The alcohol moiety metabolites formed from PBald generally follow the same pathways in mice as in rats (Ruzo et al., 1978) with some important exceptions. Only mice extensively conjugate PBacid with taurine (Hutson and Casida, 1978) and excrete glucuronides of 4′-HO-PBalc and 5-HO-PBacid. Mouse but not rat excreta contains PBald and PBalc and its glucuronide; the aldehyde appears to be less easily oxidized in mice than in rats so some is excreted per se and a portion is reduced to the benzyl alcohol. Rats but not mice convert HCN to iminothiazolidine carboxylic acid in addition to SCN^-.

Decamethrin is detoxified in mice by both oxidative and hydrolytic processes. Thus, pretreatment with either PB or DEF increases decamethrin toxicity, reduces its rate of in vivo metabolism by hydroxylation and hydrolysis, respectively, reduces the rate of product excretion, and elevates decamethrin levels in fat and brain. Decamethrin-hydrolyzing esterases are generally sensitive to organophosphate inhibition (i.e., TEPP inhibition of hydrolysis by all tissue preparations except stomach). Two factors contribute to the rapid detoxification of decamethrin: the relevant esterases are present in many tissues and the oxidases in at least liver microsomes; many molecular sites are susceptible to metabolic attack.

Poisoning of rats with 5-benzyl-3-furylmethyl (1*R*)-*cis*-chrysanthemate (White et al., 1976) and rats and birds with DDT (Hayes, 1975) is associated with critical brain concentrations of these insecticides. There are large differences in the toxicity of decamethrin to mice depending on the route of administration, the carrier vehicle, and previous exposure to PB or DEF. However, irrespective of these factors, there appears to be a critical concentration of decamethrin in brain which correlates with the onset of tremors or the time of death; further, direct intracerebral administration of decamethrin at approximately this brain level gives a similar poisoning effect. The brain appears to be a primary target in decamethrin poisoning of mice and possibly also on a more general basis in pyrethroid poisoning of mammals.

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Supplementary Material Available: Two tables giving complete data on balance studies and amounts of individual metabolites summarized in Table III (2 pages). Ordering information is given on any current masthead page.

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Comparative Metabolism of 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane and Toxaphene in Six Mammalian Species and Chickens

Mahmoud Abbas Saleh,[1] Ronald F. Skinner, and John E. Casida*

The feces of mice, rats, hamsters, guinea pigs, rabbits, monkeys, and chickens orally administered 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane contain the corresponding hexachlorobornane isomers and hexachlorobornene formed by reductive dechlorination and dehydrochlorination, respectively, at the *gem*-dichloro group. Yields of the two reductive dechlorination products are 20% in rabbits and monkeys and 3–9% in the other species and yields of the hexachlorobornene are 0.2–1.5% in each case. Toxaphene, as with the heptachlorobornane, is metabolized least extensively by chickens and most by monkeys. Most of the 29 glass capillary column gas chromatographic peaks characteristic of toxaphene are evident in the fat of all the treated animals and in the feces except for monkeys. The feces of toxaphene-treated monkeys contains the three metabolites of the heptachlorobornane discussed above as established by glass capillary column gas chromatographic comparison with authentic standards by cochromatography and electron impact mass spectrometry. Toxaphene-derived products in the liver 3 days after treatment vary with the species and in the most part are not toxaphene components.

In comparison with most pesticides, relatively little is known about the identity of the metabolites of toxaphene, a major chlorinated insecticide. This is not surprising since toxaphene is a complex mixture of related chlorinated terpenes, probably hepta-, octa-, and nonachlorobornanes in the most part, with no component making up more than 5% of the total (Saleh and Casida, 1977, 1978, 1979; Saleh et al., 1977). Some components of toxaphene are metabolized more rapidly than others, based on analyses of the fat, liver, and feces of treated rats, and new chlorinated derivatives are formed on metabolism (Saleh and Casida, 1978), a major complication in recognizing toxaphene residues in treated animals. By examining one major toxaphene component as a model, it is established that heptachlorobornane I undergoes reductive dechlorination and dehydrochlorination in rats, forming hexachlorobornanes II and III and hexachlorobornene IV, respectively (Figure 1); metabolites II–IV are less toxic than heptachlorobornane I to houseflies and goldfish (Saleh and Casida, 1978; Saleh et al., 1977).

The present investigation compares six mammalian species and chickens relative to their fecal metabolites of heptachlorobornane I and their toxaphene-derived products in fat, feces, and liver at 72 h after oral treatment. Particular attention is given to identification of three metabolites in the feces of monkeys treated with heptachlorobornane I or toxaphene.

MATERIALS AND METHODS

Chemicals. The toxaphene sample used was Lot. No. X16189-49 produced by Hercules Inc. (Wilmington, DE). Compounds I–IV were obtained as previously described (Saleh and Casida, 1978).

Treatment of Mammals and Chickens. The following test animals were treated orally with either toxaphene (~13 mg/kg) or heptachlorobornane I (~3 mg/kg): male rabbits (478–610 g) and female white leghorn chickens (1.1–1.5 kg) from Western Scientific Supply Co. (West Sacramento, CA); male Swiss-Webster mice (18–20 g), male albino Sprague-Dawley rats (150-165 g), male

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720 (M.A.S., J.E.C.), and the Finnigan Corporation, Sunnyvale, California 94086 (R.F.S.).

[1]Present address: Department of Agricultural Biochemistry, Faculty of Agriculture, University of Cairo, Giza, Egypt.

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